



Columbia Environmental Research Center

**Los Alamos National Laboratory Use Study
Phase II: Toxicity Testing of Surface Waters
and Sediment Pore Waters**

U.S. Department of the Interior
U.S. Geological Survey

Los Alamos National Laboratory Use Study
Phase II

Toxicity Testing of
Surface Waters and Sediment Pore Waters at
Los Alamos National Laboratory

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BACKGROUND AND PURPOSE

The specific uses associated with the watercourses on Los Alamos National Laboratory (LANL), presently managed by the University of California and owned by the U.S. Department of Energy (DOE), were recently contested. The Rio Grande Basin segments established by the New Mexico Water Quality Control Commission (MNWQCC) do not encompass all the perennial reaches of several streams which cross LANL. As a result, these streams are classified under the general standards, but the existing and attainable uses were contested by the New Mexico Environment Department (NMED). Rather than conducting the extensive Use Attainability Analysis, a settlement agreement allowed LANL and the DOE, with NMED, to hire a third party consultant to conduct a study for the purposes of identifying the stream uses associated with the watercourses in the canyons into which LANL discharges waters subject to NPDES regulation.

The U. S. Fish and Wildlife Service New Mexico Ecological Services Field Office (Service) submitted a Use Study Proposal (USFWS 1996) in response to the settlement agreement and to evaluate the existing and attainable uses of four watercourses on LANL. This proposal was accepted by the interested parties. The watercourses included in the Use Study are selected reaches of Cañon de Valle, Pajarito Canyon, Sandia Canyon, and Los Alamos Canyon. The proposal focused on the assessment of attainable uses, because the beneficial use of waters to support aquatic life can often require stringent numeric standards be applied to a discharge and can often be a contested use in arid regions where aquatic life can be sparse.

Four phases of use assessment were proposed: Phase I) characterization of the physical habitat; Phase II) testing of site water to determine its acute and chronic toxicity to aquatic organisms; Phase III) testing *in situ* conditions to determine any acute or chronic toxicity to caged fish; and Phase IV) determination of the survival rate of wild, native fish transplanted into the sites (Note: Phase IV of this use assessment will only commence after the review, evaluation and mutual agreement of the Selection Committee and any other interested stakeholders). The Service contracted with the US Geological Survey, Biological Resources Division, Columbia Environmental Research Center (CERC) to perform Phase II of this study. This report contains the methodology, results, and discussion of the completed Phase II (acute and chronic toxicity of site waters to aquatic organisms) portion of the use assessment. In addition, chemical analysis data of samples collected by the USFWS in conjunction with Phase III of the study is included within this report.

Specific objectives of the Phase II study were threefold: 1) determine the toxicity of site waters to the cladoceran *Ceriodaphnia dubia* (life cycle test with reproductive endpoint) and to young fathead minnows (*Pimephales promelas*; 96 hour test with lethality endpoint); 2) determine the toxicity of sediment pore waters to *C. dubia* (life cycle test with reproductive endpoint); and 3) measure water quality including metal concentrations of surface waters, pore waters, and of sediments from which pore waters were extracted.

METHODS

Site Selection

Study sites were selected by the four-person Selection Committee consisting of representatives from the NMED, DOE, and LANL, based on recommendations, flow characteristics, and other factors. Sites selected included perennial reaches of Cañon de Valle, Pajarito Canyon, Sandia Canyon, and Los Alamos Canyon below the Los Alamos Reservoir. The reference site was Los Alamos Canyon above Los Alamos Reservoir. All sites were high gradient mountain streams. Sandia Canyon, in particular, receives a large percentage of its water from Los Alamos' effluents, with at least four different Los Alamos effluents entering the stream. Sandia Canyon's watershed also contains more urban and more developed land than the other stream in the study. Sandia Canyon stream, above the selected site, flows through a lower gradient area containing a cattail marsh. No such low gradient areas apparently exist on the other streams in the study, excluding the reservoir in Los Alamos Canyon. The Cañon de Valle site was located approximately 75 m below the foot of an area which had historically served as a dump for discarded LANL equipment and materials; cleanup was proceeding on this area in 1997.

Field Procedures

Water for the surface water toxicity tests was collected daily from each site for toxicity testing as a 24-hour composite (CERC SOP F5.29). Automated samplers (ISCO Inc., Lincoln, NE) were used to collect approximately 120 mL of water every 20 minutes. The ISCO samplers pumped site water through Teflon tubing into chilled (icewater bath) 19-liter acid-washed glass containers. The composite samples were retrieved daily and transported to the mobile lab. Daily on-site measurements taken at time of retrieval of the composite sample included air temperature and dissolved oxygen (CERC SOP B4.46), pH (CERC SOP F5.33), and conductivity (CERC SOP B5.31) of *in situ* stream water. Water quality of the composite sample was evaluated at the mobile lab. Composite samples were collected daily from 8-12-96 through 8-20-96, except no sample was taken at Site 2, below Los Alamos Canyon reservoir, on 8-20-96.

Pore water (sediment interstitial water) was collected in 1996 using a method similar to that described by Winger and Lasier (1995). Fused-glass aquarium air stones attached to Teflon® tubes were inserted into depositional areas of the stream bed. Negative pressure was applied by means of a syringe, and pore water was drawn from the sediment using the glass air stone as a filter. Pore water was then injected into an acid-washed (CERC SOP C5.5) polyethylene sample bottle. The sample was then kept on ice or refrigerated until use. Several extractors were used at each site in order to obtain a sufficient total volume of pore water. Water was extracted from depositional areas along the length of the stream section that was later used by the USFWS for the Phase III *in-situ* testing with caged fish. Air stones were removed and relocated to a new

depositional area within the same site after drawing approximately 100 mL of pore water to avoid drawing overlying water through the sediment into the sample. In 1997, pore water was extracted by means of pressure filtration, using an apparatus similar to that described in Carr and Chapman (1995), but modified for portability (CERC SOP E.150). Pressure was provided by a manual pump (ambient air, ≤ 30 psi = 1550 torr). Sediment was collected from depositional areas along the same stream reach sampled in 1996. A specially designed plastic (polyvinyl chloride) scoop was used to collect sediment while introducing a minimum of surface water into the sample. The sediment was placed in a polyethylene bucket and homogenized. The homogenized sediment was then immediately used for on-site porewater extraction.

Each year, on the date of porewater extraction, two sediment samples were saved for metals analysis in glass jars, one for metals scan and the other for acid volatile sulfides and simultaneously extractable metals (CERC SOP C5.153). A third sample was saved for grain size analysis and total organic carbon analysis. In 1996, these samples were taken as grab samples from depositional areas. In 1997, these samples were subsamples of the sediment homogenate used for porewater extraction. All equipment which contacted sediment or sediment pore water was acid washed prior to use (CERC SOP C5.5).

At least three liters of pore water were collected from each site each year except no pore water was collected from the site below the reservoir at Los Alamos Canyon. There was virtually no acceptable sediment to extract at this site because of sediment entrapment by the reservoir. One hundred-mL subsamples of pore water from each site were filtered ($0.45 \mu\text{m}$) and acidified with 1% ultrapure nitric acid (v:v) and saved for metals analysis (CERC SOP C5.134). The remainder of the sample was shipped to CERC for toxicity testing.

Lab procedures

Rearing of test organisms

Ceriodaphnia dubia were reared at the CERC for more than three months prior to the tests in reconstituted soft water. Reconstituted soft water was made by addition of salts to reverse osmosis water as described by ASTM (1989), hereafter referred to as "ASTM soft." Reverse osmosis water used in making ASTM soft was derived from CERC well water passed through the reverse osmosis system and then additionally polished by a deionizer bed. Five years of analytical data on this source water have consistently shown it to be below detection for metals except for iron (mean value <0.35 mg/L) and for most elements except for sodium (mean value 5.85 mg/L), chloride (<2.00 mg/L) and sulfate (2.5 mg/L). Culture techniques were similar to those described in EPA (1994). *Ceriodaphnia* were fed a diet of fermented trout chow, yeast, and cereal leaves (YTC; EPA 1994) and algae (*Selenastrum capricornutum*). *Ceriodaphnia* used in the surface water toxicity tests were shipped overnight to LANL a month prior to the test and were maintained at LANL until the test using CERC procedures. This allowed the organisms time to recover from any ill effects of shipping.

Fathead minnows (*Pimephales promelas*) were hatched at the CERC lab in Columbia, MO, and shipped overnight to LANL the day before the test. Fathead minnow larvae were reared in CERC laboratory well water (280 mg/L hardness, 255 mg/L alkalinity, 7.8 pH). They were gradually acclimated to ASTM soft after their arrival at LANL.

Water Quality Measurements and Analytical Chemistry

Hardness, alkalinity, dissolved oxygen, pH, conductivity, turbidity, and ammonia concentration of the composite sample were measured daily at the mobile laboratory. Hardness (CERC SOP F5.95) and alkalinity (CERC SOP B4.16) were determined by titration. Dissolved oxygen was determined with a YSI® model 57 dissolved oxygen meter (CERC SOP B5.238) and conductivity was determined using a YSI® S-C-T meter (CERC SOP B 5.31). Orion® EA 940 meters (CERC SOP B.462) with glass gell electrodes (CERC SOP B4.46) were used to measure pH. The same meters with an Orion® Model 95-12 ammonia ion selective electrode were employed for ammonia determinations (CERC SOP B5.192). Turbidity was measured using a Hach® 2100A turbidimeter (CERC SOP B4.42). Calcium, chloride, sulfate, nitrate/nitrite and phosphate concentrations were measured twice during the toxicity tests using a Hach® DR 2000 spectrophotometer (CERC SOPs F5.26, F5.27, F5.28, and F5.31). Four times during the tests, (from composite samples collected on August 13, 14, 16, and 20, 1996) 100 mL subsamples of each composite sample were filtered (0.45 µm) and 1% ultrapure nitric acid (v:v) was added as a preservative for later metals analysis. A filtration blank using reverse osmosis water was prepared and preserved on each date that subsamples were preserved for metals analysis. Three separate samples of ASTM soft water were also filtered and preserved.

Water, pore water and sediment collected in 1996 and sediment collected in 1997 were analyzed at CERC for 61 elements by semiquantitative inductively couple plasma - mass spectrometry (ICP-MS), and selenium was determined by hydride-generation atomic spectroscopy. Sediments collected in 1996 and 1997 were also analyzed at CERC for acid volatile sulfides and simultaneously extracted metals (SEM-AVS). A complete listing of methods, QA/QC, and results of metals analysis at CERC may be found in Appendix A, CERC lab reports FY-97-32-08 and FY 98-32-13. Sediment and pore water collected in 1997 was analyzed by Midwest Research Institute (MRI), 425 Volker Boulevard, Kansas City, Missouri. The MRI analyzed 17 elements by a quantitative inductively coupled plasma atomic emission spectrometry (ICP) scan; for mercury by cold vapor atomic absorption spectrometry; and for selenium by hydride-generation atomic spectroscopy. MRI Narrative Report for Catalog No. 8990081, in Appendix A, includes detailed descriptions of analytical methods, applicable QA/QC information and raw data from these analyses. Sediments and waters collected by the USFWS as part of Phase III were included in this sample catalogue, and were analyzed similarly.

Sediment grain size was determined by the Bouyoucous Hydrometer Method (CERC SOP B5.179). Total organic carbon of sediment was determined in 1997 using a Coulometrics® Carbon Analyzer, Model 5020 (CERC SOP B4.36). Dissolved organic carbon of sediment pore water was measured in 1996 using a Technicon AAlI® system (CERC SOP B5.253).

In 1997 and 1998, as part of the Phase III portion of this study, USFWS provided water and sediment samples for analysis of explosives residues. These were shipped to CERC on ice, in containers wrapped in foil to minimize exposure to light. Explosives samples were analyzed at CERC, using EPA method 8330. Further explanation of the methods of analysis, QA/QC, and the list of explosives analyzed may be found CERC laboratory report FY-98-30-08, located in Appendix A, along with the results of these analyses.

In 1998, as part of the Phase III portion of the study, USFWS provided fish and sediment samples for PCB analysis. These were analyzed at CERC by capillary gas chromatography/electron capture detection methods. Further explanation of the methods of analysis, QA/QC, and the methods of PCB congener analysis may be found in CERC laboratory report FY-98-31-18, located in Appendix A, along with the results of these analyses.

Toxicity tests

Toxicity tests on surface water were performed using the cladoceran *Ceriodaphnia dubia* and also with fathead minnows. Pore water toxicity was tested with *C. dubia*. All toxicity tests were conducted using daily static renewals. A dilution series of 100, 50, 25, and 12.5% of the composite surface water or pore water was tested in all toxicity tests. EPA (1994) dictates the use of a dilution series in the case of effluent toxicity testing, but not

normally in the case of environmental samples taken outside the mixing zone. We tested a dilution series of these samples in order that, in the case of high toxicity, an estimate of the degree of toxicity could be made. ASTM soft was used as the diluent. A 100% ASTM soft control treatment was performed with each test. A positive control dilution series (i.e. reference toxicant) consisting of three concentrations of sodium chloride was also tested concurrently with each toxicity test. Lastly, a procedural control in CERC well water was also performed concurrent with each test. *Ceriodaphnia* used in the well water control were reared in well water instead of ASTM soft. An analysis of metals, minerals, and volatile organic compounds in CERC well water is included in Appendix A.

Surface Water Toxicity Testing

Toxicity tests of surface water were performed at LANL in the CERC's mobile laboratory. Because of the difficulties in sample retrieval associated with these mountainous sites, it was impossible to start the test on the same day as water collection. Therefore, each day's composite samples were held overnight (after water quality measurement) before use in toxicity testing on the following day.

Ceriodaphnia dubia toxicity test was conducted according to USEPA (1994). Animals were exposed to 20 mL of the composite water sample or the appropriate dilution in 30-mL glass beakers for seven days. One neonate, less than 12 hours old, was added to each beaker at the beginning of the test (day 0). There were ten replicates of each treatment. Endpoints, recorded daily, were lethality (absence of movement) and reproduction (number of neonates produced). Temperature in the test beakers was maintained at $20 \pm 1^\circ\text{C}$ by means of a temperature controlled water bath. In addition to the water quality parameters measured daily on the water used in the test, test and control waters from exposure beakers (post-exposure) was collected for dissolved oxygen and pH measurements. Ammonia concentration was also measured on the post-exposure water on day 5 and day 7. In order to have sufficient volume of post-exposure sample, water from all replicates within a treatment was composited in a 250-mL beaker for DO, pH, and ammonia analysis.

In response to a mortality event on day three of the surface water toxicity test with *Ceriodaphnia dubia*, a second toxicity test was started on 8-15-96. This test was similar to the first toxicity test, except no dilutions of the site waters were tested, and the duration was only 120 hours. Because of the short duration of this test, no reproduction data was obtained.

The fathead minnow toxicity test was conducted according to USEPA (1993) and ASTM (1989) protocol for acute toxicity testing. The test was started on 8-14-96. Fish were less than 72 hours post-hatch at the start of the test. Test containers were 1 L beakers containing 0.75 L of composite sample or appropriate dilution. Ten fish were stocked per container. Four replicates of the 100% concentration of each site and two replicates of each dilution concentration were tested. Fish were fed brine shrimp (*Artemia* sp.) nauplii (≤ 24 h old) twice daily. Test duration was 96 hours. The endpoints, recorded

daily, were lethality (i.e. animal does not move with gentle prodding) and moribundity (i.e. animal does not retain equilibrium or does not swim normally until prodded).

Water quality (temperature, dissolved oxygen, pH, conductivity) were measured daily in fathead minnow test chambers. Adequate oxygen levels were maintained in test chambers by continuous gentle aeration. All chambers were inspected daily for moribund or dead organisms. Dead organisms were removed during water change. Temperature in the chambers was maintained at $20 \pm 1^\circ\text{C}$ by controlling ambient temperature in the mobile lab.

Porewater toxicity testing

Porewater toxicity tests were performed with *Ceriodaphnia dubia*. Methods used were equivalent to those used to test surface water, except that pore water was collected as a single pooled sample from each site as opposed to daily collections as for surface water. The pooled sample was shipped to CERC for toxicity testing, where it was centrifuged to remove fines (CERC SOP B4.64). Maximum holding time between collection of pore water at LANL and the start of toxicity test at CERC was four days in 1996 and ten days in 1997. In 1997, the sample from Site 1 (Los Alamos Canyon above the reservoir) was inadvertently contaminated prior to the test. This sample was recollected and retested four weeks later, using a separate but equivalent set of controls. In response to toxicity in some 12.5% dilutions of pore water, the 12.5% dilutions were retested (concurrent with the second test with Los Alamos water) using ASTM soft as the dilution water, and with a second reconstituted water of similar hardness made by diluting well water with reverse osmosis water.

Logistic analysis (Agresti 1990) was used to evaluate survival data. Analysis of variance (Snedecor and Cochran 1989) was used to evaluate reproduction data. In the analysis of variance, total reproduction for each ceriodaphnid over the seven day test (including those that died during the experiment) was used as the dependent variable. Dunnett's method was used to control the experimentwise error rate at $\alpha = 0.05$ for comparing reproduction in site waters to control. Tukey's method (Hochberg and Tamhane 1987) was used to control experimentwise error rates at $\alpha = 0.05$ in the comparison of reconstituted water dilutions.

RESULTS and DISCUSSION

Water Quality and Sediment Parameters

In situ water quality

Dissolved oxygen, pH, temperature, and conductivity values measured in-situ are given in Appendix B, Table A. Lowest dissolved oxygen values were consistently measured at

Sandia Canyon. However, the lowest concentration measured was 5.6 mg/L, which is sufficient to maintain most forms of aquatic life. Oxygen concentrations at other sites ranged from 6.4 to higher than 8 mg/L. Neutral pH values were recorded at all sites and all dates. Los Alamos Canyon below the reservoir had slightly reduced pH values compared to the stream above the reservoir. *In situ* measurements were generally taken in the morning, especially at this site; thus the pH difference was likely due to community respiration within the reservoir. Had pH been measured in the afternoon, this may have been reversed due to photosynthesis. Temperatures ranged from 12 to 16.5 °C. Conductivity varied between streams, with Los Alamos Canyon being the least conductive, followed by Pajarito, then Cañon de Valle, and then by Sandia Canyon, which was very conductive (Figure 1). Conductivity within streams was remarkably stable, except at Sandia Canyon and on one date at Cañon de Valle.

Water quality of daily composite samples

Daily water chemistry measurements taken on the composite samples are given in Appendix B, Tables B and C. Mean values are given in Table 1. As is typical of Rocky Mountain streams, all waters were soft, high in dissolved oxygen, and low in ammonia. As in the measurements taken *in situ*, composite samples from Sandia Canyon were atypical in water quality. In particular, Sandia Canyon was much higher in conductivity (Table 1) and in nutrients, chlorides, and sulfates (Table 2). The very high chloride values at Sandia Canyon account for the disparity between the high conductivity and only moderately elevated hardness at that site. All Pajarito Canyon water samples were slightly milky in color and were generally higher in turbidity, regardless of rainfall events (Figure 2). The source of this milky color is not known. Whereas most water quality values did not vary considerably over the duration of the toxicity testing period, rains in the watershed did cause variation in the turbidity of the composite sample (Figure 2). A widespread rain raised the turbidity of most the streams on 8/14/96, and localized heavy rain raised the turbidity of Sandia Creek on three other days.

Quality of pore water

As with the surface water samples, pore water from Sandia Canyon differed from the other sites in its higher concentration of nutrients, sulfates, chlorides, and had higher hardness and alkalinity (Table 3). All sites had neutral pH values, and ammonia concentrations were low at all sites, although somewhat higher at Sandia than at the others. The Los Alamos Canyon site below the reservoir was not sampled either year because there was no appropriate sediment at this site, due to sediment trapping by the reservoir immediately upstream. The Los Alamos site above the reservoir was sampled twice in 1997, because of an inadvertent contamination of the original sample. The toxicity of this site was retested on water from the second sampling (Table 3, Los Alamos b). Pore water from the second 1997 sampling at the Los Alamos site was generally similar to the first 1997 sampling, but contained a lower calcium concentration.

Sediment parameters

The finest sediments available were selected for metals analysis and for porewater extraction. However, in these high gradient streams, even the most depositional areas were mostly sand (Table 4). Organic material in the environment sequesters organic contaminants making them less bioavailable (Gobas and Zhang, 1994). All sediments in this study were low in organic carbon, (Table 4) as might be expected in mountain streams and in sediments of such large grain size. Therefore organic contaminants in these streams would likely be highly available to resident organisms. Los Alamos Canyon was somewhat higher in TOC compared to the other streams. This may have been due to its higher altitude and therefore higher annual precipitation. Plant growth was much more lush in this canyon than in the others.

Metals concentrations

Complete raw data of metals concentrations in sediments and water and associated QA/QC blanks may be found in Appendix A. MRI's extraction of Phase II and III water samples and metals analysis of the extracts was completed within the maximum holding time mandated by the Clean Water Act, but then a cracked mirror was observed in the spectrophotometer (personal communication, Gary Wester, MRI). The machine was repaired and the same extracts were reanalyzed. However, this second analysis did not fall within the specified holding period. The sample extracts should not have degraded, and should be good for years (personal communication, John Moore, Patuxent Analytical Control Facility, US Geological Survey). However, because of the legal ramifications of the Clean Water Act holding time limitations, both sets of data are given in Appendix A, Table A. The relative percent difference between the two analyses, for each element, is given in Appendix A, Table B. Sediment samples are not affected by this holding time limit.

Tables 5 and 6 give results (second analysis, with the repaired instrument) of the quantitative analysis of sediment and sediment pore water performed on 1997 samples collected as part of Phase II. Results of 62 element semi-quantitative scans of surface water, pore water, and sediments can be found in Appendix B, Tables D, E, and F respectively. Some elements were elevated compared to normal background concentrations, especially barium and strontium in Cañon de Valle and molybdenum and strontium in Sandia Canyon. These compounds were elevated in surface water, pore water, and sediments at those sites (Table 5 and 6, Appendix B tables D, E, and F). However, these are not highly toxic elements and they are not present at concentrations likely to cause toxicity (USEPA 1986, Nadi et al. 1995). Porewater concentrations of chromium at Sandia Canyon and cadmium at all sites were elevated in the MRI's metals analysis (Table 6). These concentrations exceed the minimum concentrations which have been shown to have reproductive effects on daphnids in very soft waters, especially for cadmium. Cadmium in very soft water has been shown to have reproductive effects on daphnids at concentrations as low as 0.17 $\mu\text{g/L}$ (Beisinger and Christensen 1972), and the water quality criteria (at 50 mg/L hardness) for cadmium is 0.66 $\mu\text{g/L}$ (USEPA 1986). However, it should be noted that MRI lab blanks also

contained measurable cadmium (up to 3 $\mu\text{g/L}$) and chromium (up to 7 $\mu\text{g/L}$) suggesting that the values of these two elements may be elevated over the actual field concentration. Cadmium concentrations in the MRI's analysis of sediment were not highly elevated, further evidence that the elevation of cadmium in surface and pore waters may be artifactual. Furthermore, the CERC's semiquantitative analysis of a split of the same sediment sample did not find detectable concentrations of cadmium (Appendix B, Table D).

Acid volatile sulfides (AVS) sequester certain metals (simultaneously extractable metals; SEM) which controls their bioavailability (Di Toro et al., 1992). An SEM/AVS ratio greater than one indicates that metals should be bioavailable. The AVS in LANL sediment samples was generally low, resulting in high availability of metals, especially in 1996 and in Pajarito Canyon both years (Table 7). However, overall concentrations of bioavailable SEM (expressed as SEM - AVS) were probably not high enough to cause toxicity (Table 8). AVS minus simultaneously extractable cadmium values were negative at all sites and times except 1997 at Pajarito Canyon, (Table 8) further indication that cadmium concentrations were not likely the cause of toxicity observed in Sandia Canyon pore water.

Toxicity testing

Surface water tests

The surface water test with fathead minnows was begun on 8/14/96 with water collected on 8/13/96. The test was terminated on schedule on 8/18/96. No toxicity was observed in the fathead minnow toxicity test. Mortality did not exceed 10 percent in any undiluted treatment, and mortality in the dilution treatments did not exceed 20 percent. (Appendix C, Table A). Moribundity was also very low in all treatments. In one replicate of one treatment (Sandia Canyon, 25% dilution) forty percent moribundity was observed, but there were no moribund animals and 100% survival in the paired replicate. A power outage occurred on 8/17/96. Although aeration and temperature control were temporarily lost, temperature of the chambers did not exceed 23°C and dissolved oxygen concentrations did not drop below 6 mg/L. Water quality data on the fathead minnow toxicity test is located in Appendix C, Table B.

The *Ceriodaphnia dubia* toxicity test with surface water was begun on 8/13/96 with water collected 8/12/96 and terminated on schedule on 8/20/96. Survival and reproduction of organisms exposed to the undiluted samples are illustrated in Figure 3. Complete survival and reproduction data from this test are found in Table 9. Complete mortality occurred in the undiluted sample from Cañon de Valle on day three of the study. Survival was very high and reproduction was normal in all other undiluted treatments. Survival and reproduction in Cañon de Valle water increased with increasing dilution of the sample water (Figure 4). The mortality in Cañon de Valle appeared to be associated with a rain event on 8/14/96. There was widespread rain over most LANL watersheds on that date, as evidenced by the increased turbidity of that day's composite samples (Figure 2). In response to this event, a second *Ceriodaphnia* toxicity test was started on 8/15/96, using only undiluted sample, to see if the toxicity recurred. This test was terminated on day 5, 8/20/96; survival to that date was 88.9% (one mortality of nine individuals) in Cañon de Valle water. Survival was 100% in all other treatments in this second, short, test. The second test was aborted on day five because of logistical problems, too early to evaluate reproduction.

Although some metals were found in elevated concentrations in water, pore water, and sediments, it appears unlikely that these metals are responsible for the observed toxicity unless they were in some way activated or temporarily increased in concentration by the rain event. Unfortunately, although water was sampled four times for metals analysis during the study, none of these samples fell on the day which apparently caused the toxicity. Metals samples must be filtered and acidified soon after collection, and due to the nature of the test, toxicity was not recorded until nearly 48 hours after the sample collection.

During the Phase III portion of this test, PCBs and explosives were measured in sediment and water. Cañon de Valle had elevated concentrations of explosives, notably HMX, RDX, 4-Am-2,6-DNT, and 2-Am-4,6-DNT (Table 10). Although these compounds are toxic, they are not likely to have caused the extreme acute toxicity

observed at Cañon de Valle. The measured concentrations of RDX at Cañon de Valle were at least two orders of magnitude below concentrations which caused the reproductive endpoint toxicity (Peters et al, 1991). In fact, solubility limits might preclude RDX from exhibiting observed toxicity. HMX is somewhat less soluble than RDX, and less toxic to the bacteria *Vibrio fischeri* than RDX (Drzyzga, 1995). However, it is possible that *Ceriodaphnia* may have consumed particulates in the treatment water resulting from the runoff from the rain event. This would have resulted in a strong increase in the effective dose. There is no available data on this type of explosives exposure to daphnids. The area immediately upstream of the site has historically been used as a dump. The contents of the dump are not known, but pipes and steel drums were visible in the dump. In 1997, during cleanup of this area, explosives were found within the dump (Joel Lusk, USFWS, personal communication). Runoff from the rain event may have carried higher concentrations of explosives or some unknown contaminants into the stream.

Pronounced toxicity was observed in the lowest dilution of surface water from Sandia Canyon (100% mortality) and in all three dilutions of water from Pajarito Canyon (80, 100, and 100% mortality in the 50, 25, and 12.5% dilutions, respectively). Higher concentrations of water from Sandia Canyon and the undiluted water from Pajarito Canyon were not toxic. The reason for this apparent reverse toxicity is unknown; dilutions were made with the same ASTM soft that caused no toxicity and with which all other treatments were compared. All mortality except one individual (Pajarito Canyon 50% dilution) were dead on day three, the same day the mortality occurred in the Cañon de Valle 100% treatment. There is no evidence of any artifactual cause of this mortality; toxic samples were not grouped together in the waterbath, and the dilution water showed no toxicity. The power outage on 8/17/96 occurred well after the toxic event, and did not affect the temperature of the test, which was buffered against temperature change by the size of the water bath.

When water was renewed daily, the old water from each treatment was saved. All beakers from any replicate were pooled to have sufficient sample for dissolved oxygen measurement. Oxygen concentrations on this water remained above 6.8 mg/L in all cases. Total Ammonia - Nitrogen, measured in these waters on 8/18/96 and 8/20/96, did not exceed 0.7 mg/L in any case.

Sediment porewater toxicity testing

The toxicity test performed on pore water collected in 1996 failed due to the presence of male *Ceriodaphnia* in the test. Male *Ceriodaphnia* are indistinguishable from females when less than 24 hours old. Unstressed females generally reproduce parthenogenetically, producing only female clones. When stressed, they will produce male, sexually reproducing, young. The presence of a significant number of males in a test is an indication that the animals were taken from a stressed culture, and the test may yield invalid results. Therefore, the test was repeated with new porewater samples in 1997.

The results of the 1997 porewater toxicity test with undiluted pore water are illustrated in Figure 3, and data for all dilutions is found in Table 11. Reproduction was significantly reduced, compared to the ASTM soft control, in animals exposed to pore water from Sandia Canyon. Survival was slightly lower at that site. Los Alamos Canyon pore water in 1997 was inadvertently contaminated prior to the test, therefore this site was resampled and retested. Reproduction in animals exposed to pore water from Los Alamos Canyon was significantly higher than the ASTM soft control in this second test.

As discussed above, metals found at Sandia Canyon are not likely to be the cause of the observed toxicity. In Phase III of this study, polychlorinated biphenyls (PCBs) were found to be somewhat elevated (up to 155 $\mu\text{g/kg}$) in Sandia Canyon sediments. These values approach levels which have been associated with toxicity in sediment quality assessments. Smith et al. (1996) used a large database of sediment toxicity tests to calculate threshold effects levels (TEL; geometric mean of the fifteenth percentile of a data set of toxic PCB contaminated sediments and the fiftieth percentile of the a non-toxic data set) and probable effects levels (PEL; geometric mean of the fiftieth percentile of the toxic data set and the 85th percentile of the non-toxic data set) for freshwater sediments. The TEL is intended to identify the sediment concentration for a chemical below which sediment concentrations rarely occurred, and the PEL is intended to estimate the sediment concentration for above which adverse biological effects frequently occurred. These values are not adjusted for organic carbon concentrations in the sediment. The TEL for total PCB was 34.1 $\mu\text{g/kg}$ and the PEL was 277 $\mu\text{g/kg}$. Sandia Canyon sediments are low in organic carbon, and therefore the activity of PCB in these sediments may be assumed to be somewhat higher than normal. Sandia Canyon's sediment PCB concentrations fall between these two concentrations and therefore into the potentially toxic range.

The reverse toxicity which occurred in the surface water test was found again in the porewater test, although this time not as strongly, and it was confined to the 12.5 percent dilutions. Reproduction and survival was reduced in 12.5% concentrations of pore water from Sandia, Pajarito, and Cañon de Valle (Table 11). To investigate this phenomenon further, the 12.5% dilutions were retested (concurrent with the second test with Los Alamos water) using ASTM soft as the dilution water, and with a second reconstituted water of similar hardness made by diluting well water with reverse osmosis water. In this test, survival did not differ between site water/soft water combinations (Table 12). However, significant differences in reproduction were detected between dilution waters at Pajarito Canyon and Cañon de Valle. Mean reproduction in dilutions with soft water made from well water was always higher than in dilutions made with ASTM soft at each site, although not significantly at Los Alamos Canyon and Sandia Canyon. The interaction between site water and soft water preparation was significant ($p < 0.021$), therefore the effect of soft water preparation on reproduction depended on the site water. The nature of the interaction between ASTM soft water and the site water is unclear. However, it is clear that some kind of interaction did occur.

Mortality in the surface water tests occurred suddenly on day three, both in undiluted water and in the case of the reverse toxicity. In the porewater tests, mortality was not as

severe and was spread out over the course of the test. This is to be expected; pore water was collected all on the same day and daily aliquots of the sample were used for testing. Also, porewater toxicity should not rise and fall sharply on a daily basis. Short term events which cause toxicity in surface water should effect porewater toxicity, like sediment toxicity, only slightly. The net effect should be an integration of rapid daily changes in toxicity of the overlying water.

For reference, results of the 1996 porewater toxicity test are given in Table 13. No statistics were performed on this data because of the problems associated with the test and because of low numbers of replicates in some treatments after the exclusion of replicates containing males. The data in Table 13 should be interpreted with care because this test is invalid due to the presence of males and replication of some treatments is low. However, the results of the 1996 and 1997 porewater toxicity tests were remarkably similar once the influence of males was excluded from the data. Reproduction in pore water from Sandia Canyon was reduced both years, and survival was also lower in that treatment. Reproduction was higher than the ASTM Soft control in pore water from Los Alamos Canyon both years. Reverse toxicity was apparent in the 12.5% dilution of some pore waters in both years.

CONCLUSIONS

Water quality variables, including dissolved oxygen, hardness, alkalinity, temperature, pH, turbidity, ammonia, sulfate, nitrate/nitrite, chlorides, and calcium, were clearly within acceptable for fish and stream invertebrates. Sandia Canyon stream was somewhat unusual in its water chemistry compared to most mountain streams and to the other streams in this study. However, the water quality variables measured there are acceptable values for fish and invertebrates. Some metals were elevated above background at some sites, however, concentrations do not seem to be high enough to cause the toxicity observed.

Strong toxicity to *Ceriodaphnia dubia*, possibly due to the influence of a rain event, was observed in surface waters from Cañon de Valle. Elevated concentrations of explosives were identified in Cañon de Valle sediment and water as part of the Phase III portion of this study. Runoff from the rain event may have temporarily increased the concentration of explosives or it is possible that some other unidentified contaminant in runoff was responsible for this toxicity. No toxicity to fathead minnows was observed at any site. The fathead minnow test was in progress on the day that caused severe toxicity in the *Ceriodaphnia dubia* toxicity test, and the same water was used in both tests. However, fathead minnows were apparently not adversely effected by water which caused complete mortality in *Ceriodaphnia*.

Pore water from Sandia Canyon was moderately toxic to *Ceriodaphnia dubia*. The observed toxicity cannot be positively explained by any of the measured water quality variables, metals concentrations, or explosives analyses. Sediment PCB concentrations were moderately high and may be wholly or partially responsible for the observed toxicity

at Sandia Canyon. However, this site receives a number of complex effluents. A complete toxicity identification analysis would be required to identify the source of this toxicity.

The reason for the reverse toxicity in some dilutions of surface and pore water, rather than in the undiluted sample, remains unknown. It is therefore impossible to tell if this contains any ecological or toxicological significance in Los Alamos streams. However, mixing some site waters or pore waters with ASTM soft water (essentially a mixture of reverse osmosis water and common salts) appeared to create a toxic interaction.

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FIGURES

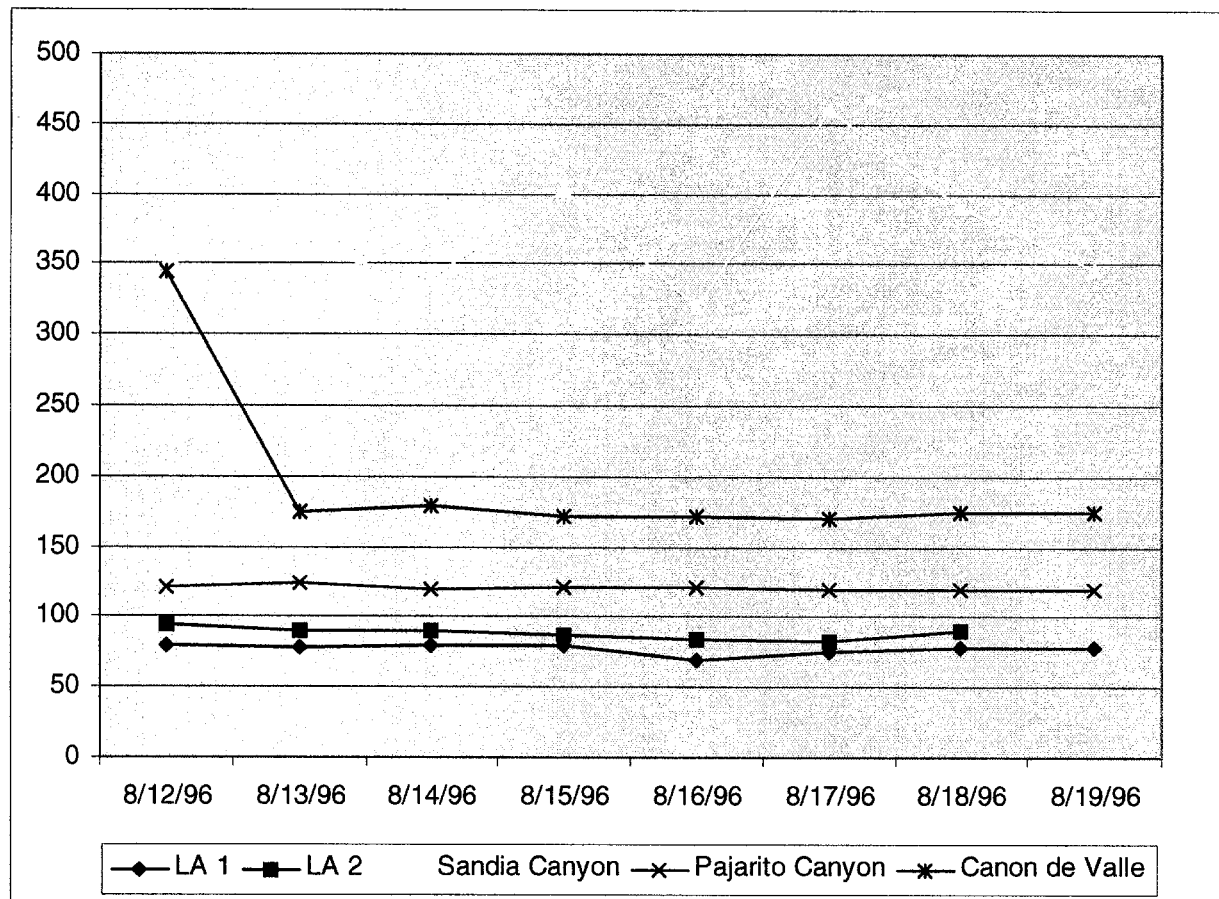


Figure 1. *In situ* conductivity ($\mu\text{mhos/cm}^3$) of Los Alamos National Lab streams. LA 1 and LA 2 indicate Los Alamos Canyon above and below the reservoir, respectively.

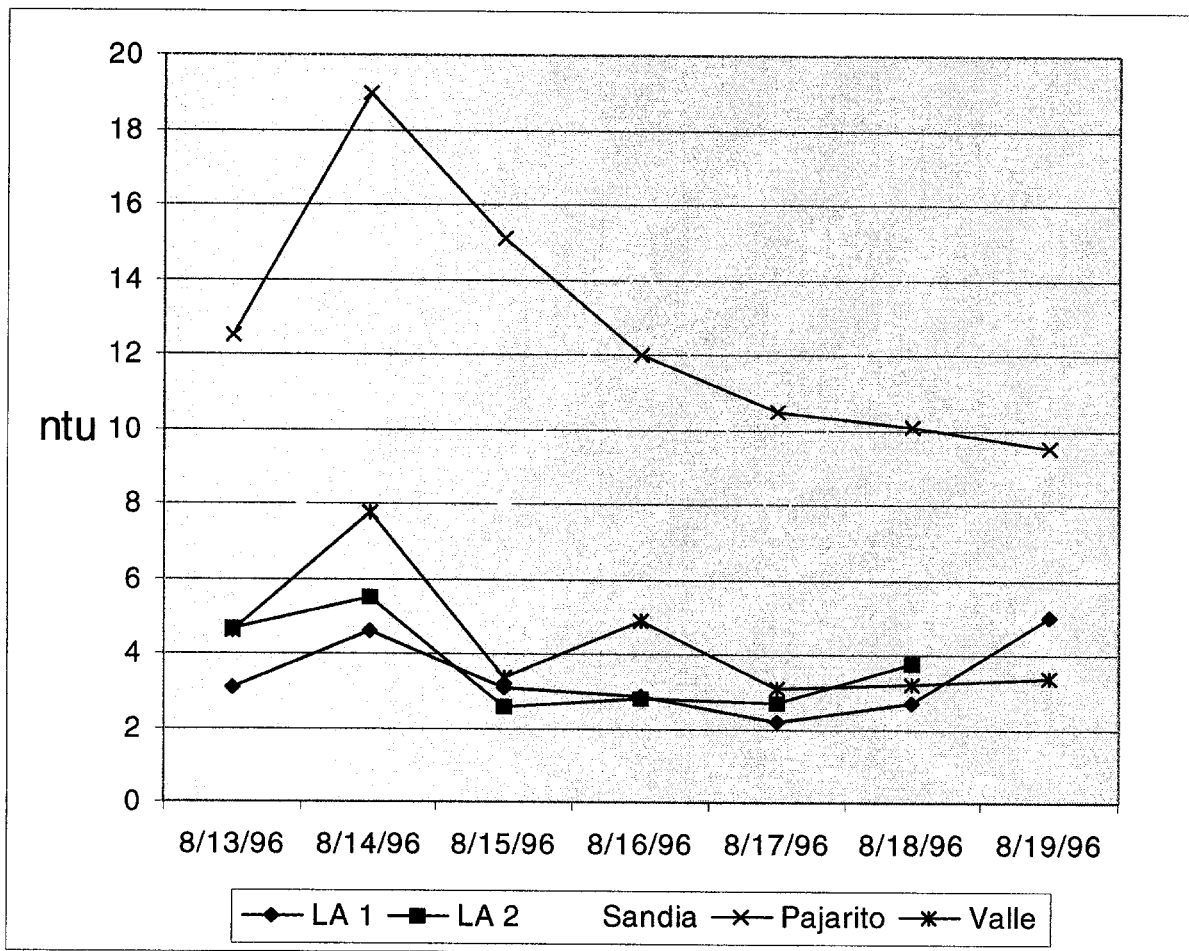


Figure 2. Turbidity of daily composite samples. LA 1 is the Los Alamos Canyon site above the reservoir, and LA 2 is below the reservoir. The Sandia Canyon sample on 8/19/98 was off chart, at 35 ntu.

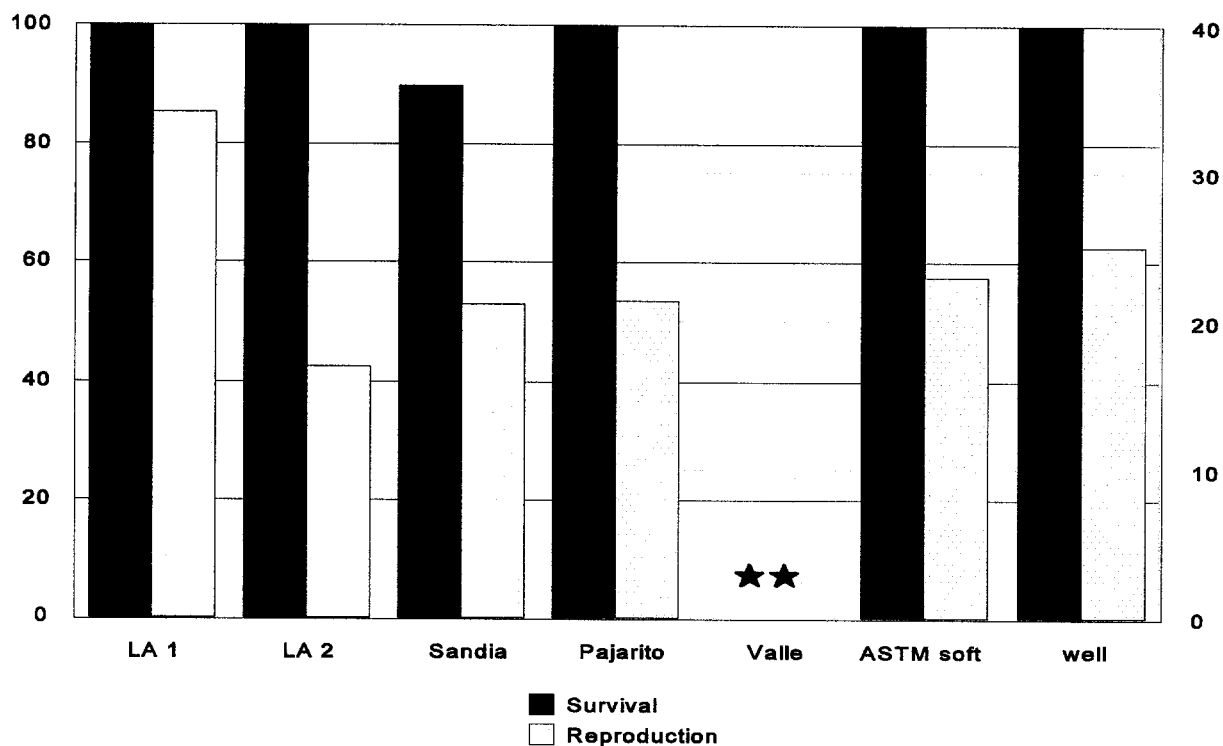


Figure 3. Survival and reproduction of *Ceriodaphnia dubia* exposed to undiluted daily 24 hour composite samples of surface water from streams located at Los Alamos National Lab. LA 1 and LA 2 are Los Alamos Canyon above and below the reservoir, respectively. Stars indicate a significant difference from ASTM soft.

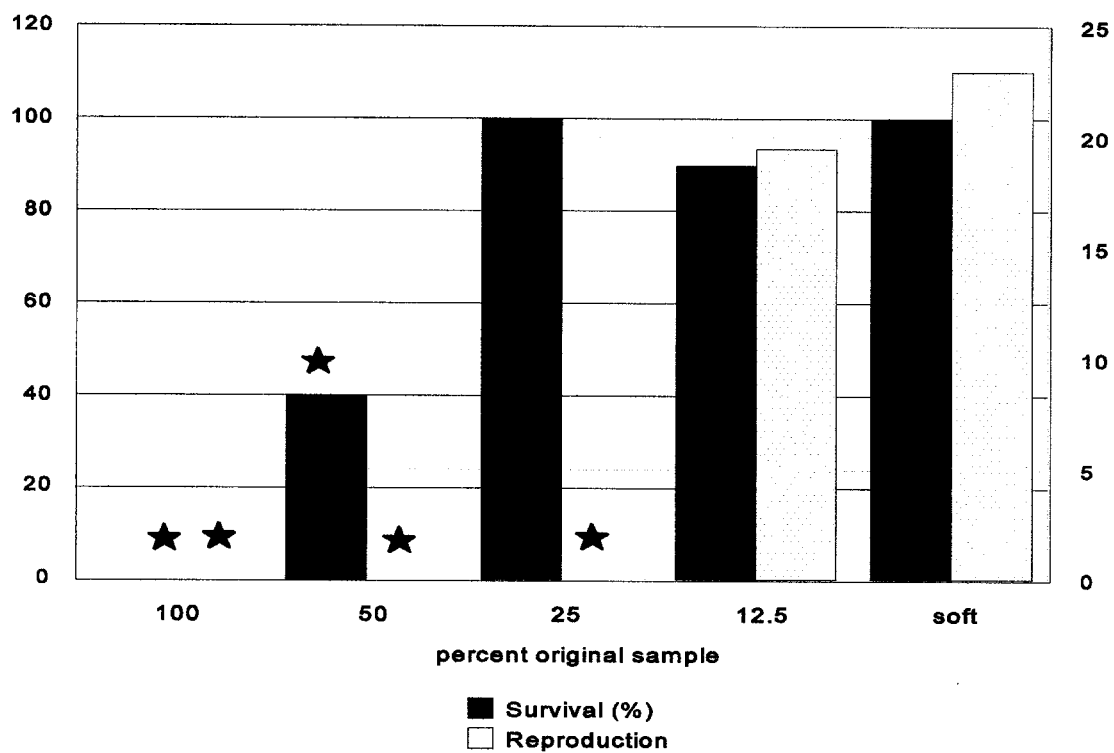


Figure 4. Survival and reproduction of *Ceriodaphnia dubia* exposed to full strength and three dilutions of daily 24 hour composite samples of surface water from Cañon de Valle. Stars indicate a significant difference from ASTM soft.

Tables

Table 1. Mean water quality values of daily composite samples. Parameters were measured daily during 1996 toxicity testing of streams at Los Alamos National Lab. Well water and ASTM Soft water were transported from Columbia Missouri in 5 gallon carboys, and a subsample of the carboy in use was measured daily.

Site	pH	Conductivity (μ mhos/cm ³)	DO (mg/L)	TAN ¹ (mg/L)	Alkalinity (mg/l as CaCO ₃)	Hardness (mg/l as CaCO ₃)	Turbidity (ntu)
Well	8.16	493	6.0	0.059	198	223	0.2
ASTM Soft	7.80	166	6.0	0.053	41.2	49.7	1.0
Los Alamos 1	7.77	98	7.0	0.036	39.2	32.6	3.1
Los Alamos 2	7.64	104	6.9	0.048	41.5	35.6	3.7
Sandia Canyon	7.89	432	7.1	0.053	132	79	9.9
Pajarito Canyon	7.76	149	7.4	0.031	46.6	46.7	13.2
Cañon de Valle	7.86	216	7.2	0.032	82.3	66.9	4.5

¹Total ammonia-nitrogen

Table 2. Water quality of composite samples from streams at Los Alamos National Lab. Values are means of two measurements during 1996 toxicity testing. Well water and ASTM Soft were transported from Columbia Missouri in 5 gallon carboys, and a subsample of the carboy in use was measured daily. All values are mg/L.

Water source	NO ₂ /NO ₃	SO ₄	PO ₄	Cl	Ca
Well	0.08	59.5	0.02	21.3	57
ASTM Soft	0.05	37.5	0.01	1.7	16
Los Alamos 1	0.07	3	0.19	8.8	28
Los Alamos 2	0.05	3	0.17	7.3	22
Sandia Canyon	0.29	47	11.4	38	37
Pajarito Canyon	0.17	7	0.14	14.8	36
Cañon de Valle	0.08	7	0.17	14.7	41

Table 3. Water chemistry of sediment porewater collected from streams at Los Alamos National Lab. No porewater was collected from the Los Alamos site below the reservoir because insufficient sediment existed at this site. All values except pH are in mg/L, hardness and alkalinity expressed as mg/L as CaCO₃.

Year	Site	pH	chlorides	PO ₄	NO ₂ /NO ₃	TAN ¹	Ca	hardness	alkalinity	TOC ²	SO ₄
1996	Los Alamos 1	7.57	3.6	0.23	0.16	0.05	2	34	36	3.42	nm ³
	Sandia Canyon	7.90	7.0	12.4	0.27	0.19	3	90	112	4.05	nm
	Pajarito Canyon	7.45	2.2	0.09	0.12	0.05	3	54	56	7.23	nm
	C. de Valle	7.63	5.6	0.39	0.08	0.05	3	72	48	3.98	nm
1997	Los Alamos 1	7.39	12.7	0.15	0.11	0.05	24	32	35	nm	3.7
	Sandia Canyon	7.78	59.6	4.25	0.07	0.13	8	98	154	nm	120
	Pajarito Canyon	7.18	14.6	0.12	0.07	0.05	8	38	43	nm	4.9
	C. de Valle	7.57	14.6	0.29	0.10	0.05	20	62	82	nm	6.8
	Los Alamos 1b ⁴	7.47	8.1	0.09	0.08	0.05	4	30	40	nm	6.5

¹Total ammonia - nitrogen

²Total organic carbon

³Not measured

⁴Repeat sampling of Los Alamos site

Table 4. Grain size and total organic carbon concentration of sediments collected for metals analysis and for porewater extraction from streams located on Los Alamos National Lab. No sediment was collected from the Los Alamos Canyon site below the reservoir because insufficient sediment existed at this site.

site	sand %	silt %	clay %	TOC ¹ ($\mu\text{g/g}$)	date sampled
Los Alamos 1	90.8	6.0	3.2	nm	8/20/96
Sandia Canyon	88.8	7.3	3.9	nm	8/21/96
Canon de Valle	90.1	6.6	3.3	nm	8/21/96
Pajarito Canyon	88.4	8.0	3.6	nm	8/21/96
Los Alamos 1	92.1	6.1	1.9	7167	8/14/97
Sandia Canyon	90.2	6.0	3.8	4756	8/11/97
Canon de Valle	89.3	7.0	3.6	3738	8/13/97
Pajarito Canyon	87.5	9.1	3.4	4142	8/12/97
Los Alamos 1b	91.2	5.5	3.3	7675	9/17/97

¹Total Organic Carbon

Table 5. Results of metals analysis of sediments collected in 1997 from streams located on Los Alamos National Lab. All results in mg/kg.

	Los Alamos Canyon	Sandia Canyon	Pajarito Canyon	Cañon De Valle
Aluminum	5068.60	6668.70	8034.40	6330.80
Arsenic	1.74	1.70	1.70	1.70
Barium	40.67	69.68	81.22	803.55
Beryllium	0.65	0.56	0.70	0.54
Boron	1.91	3.05	2.02	3.01
Cadmium	0.16	0.35	0.44	0.16
Chromium	4.16	183.43	7.58	5.85
Copper	3.03	13.36	9.56	11.66
Iron	4803.20	9358.60	9804.50	8199.30
Lead	21.39	15.71	14.64	19.58
Magnesium	556.20	945.27	1158.80	991.45
Manganese	231.65	397.59	558.05	308.99
Mercury	0.10	0.11	0.12	0.08
Molybdenum	0.73	2.15	0.74	0.65
Nickel	4.25	5.03	12.83	5.72
Selenium	0.26	0.26	0.35	0.25
Strontium	8.72	10.32	13.48	9.14
Vanadium	6.34	10.65	15.54	10.03
Zinc	26.83	99.32	34.44	47.15

Table 6. Results of metals analysis of sediment pore waters collected in 1997 from streams located on Los Alamos National Lab. All results in $\mu\text{g/L}$.

	LA 1	LA 1 b	Sandia	Pajarito	Valle
Aluminum	115.20	372.70	251.54	200.72	98.79
Arsenic	21.53	21.53	21.53	21.53	21.53
Barium	28.96	29.40	76.94	130.25	3579.50
Beryllium	0.41	0.42	0.37	0.51	0.37
Boron	19.27	19.27	68.26	19.27	37.12
Cadmium	4.36	4.14	4.91	5.36	3.81
Chromium	7.17	6.51	16.42	10.48	8.04
Copper	4.91	5.45	9.45	11.52	7.85
Iron	39.30	120.73	173.30	58.12	43.83
Lead	15.87	15.87	15.87	15.87	15.87
Magnesium	2889.40	2841.80	6204.10	3056.00	4894.20
Manganese	127.25	68.62	1181.40	613.31	811.17
Molybdenum	3.97	3.97	53.28	3.97	3.97
Nickel	11.44	10.14	16.38	19.13	14.48
Selenium	2.60	2.60	2.60	2.60	2.60
Strontium	56.81	60.59	107.22	63.83	121.22
Vanadium	5.32	3.89	10.71	9.59	5.84
Zinc	14.99	16.48	24.83	24.91	17.02

Table 7. SEM÷AVS, calculated as simultaneously extracted metals $\mu\text{Mol/g}$ divided by acid volatile sulfides, $\mu\text{Mol/g}$, of sediments collected from streams located on Los Alamos National Lab.

year	site	Cd	Cu	Ni	Pb	Zn	Σ^1
1996	LA 1	0.048	1.69	1.74	3.72	14.6	21.8
	Sandia	0.302	10.8	4.87	5.13	89.7	111
	Pajarito	0.331	9.13	4.91	16.08	36.6	67.1
	Valle	0.337	51.0	4.35	11.5	86.0	153
1997	LA 1	0.002	0.076	0.056	0.160	0.54	0.83
	Sandia	0.002	0.068	0.014	0.026	0.53	0.64
	Pajarito	0.236	3.77	4.17	5.71	5.50	19.3
	Valle	0.010	2.00	0.259	0.510	1.96	4.74

¹ $\Sigma = \Sigma[\text{Cd, Cu, Ni, Pb, Zn}] \mu\text{Mol/g} \div \text{AVS } \mu\text{Mol/g}$.

Table 8. SEM-AVS calculated as simultaneously extracted metals $\mu\text{Mol/g}$ minus acid volatile sulfides $\mu\text{Mol/g}$ of sediments collected from streams located on Los Alamos National Lab.

year	site	Cd	Cu	Ni	Pb	Zn	Σ^1
1996	LA 1	-0.003	0.0021	0.0023	0.0084	0.0421	0.64
	Sandia	-0.0015	0.0216	0.0085	0.0091	0.1952	0.242
	Pajarito	-0.0009	0.0114	0.0055	0.0211	0.0499	0.093
	Valle	-0.0017	0.13	0.0087	0.0274	0.221	0.396
1997	LA 1	-0.1	-0.1	-0.1	-0.1	-0.1	-0.02
	Sandia	-0.8	-0.7	-0.8	-0.7	-0.4	-0.3
	Pajarito	0.01	0.03	0.04	0.1	0.1	0.2
	Valle	-0.1	0.1	-0.05	-0.03	0.1	0.2

$^1\Sigma = \Sigma[\text{Cd, Cu, Ni, Pb, Zn}] \mu\text{Mol/g} - \text{AVS } \mu\text{Mol/g}.$

Table 9. Reproduction and survival in *Ceriodaphnia dubia* exposed to daily composites of surface waters collected in 1996 from Los Alamos National Lab streams. N indicates the number of replicates; some treatments had less than ten replicates because of handling mortality during the test.

Site	dilution (% original sample)	survival (%)	mean reproduction	N
Los Alamos Canyon 1	100	100	34.5	8
	50	88.9	24.3	9
	25	90	25.0	10
	12	80	29.6	10
Los Alamos Canyon 2	100	100	17.8	9
	50	100	22.125	8
	25	80	19.2	10
	12	60	19.0	10
Sandia Canyon	100	90	21.2	10
	50	88.9	18.4	9
	25	90	20	10
	12	0	0	10
Pajarito Canyon	100	100	21.4	10
	50	20	3.4	10
	25	0	0	10
	12	0	0	10
Cañon de Valle	100	0	0	9
	50	40	0	10
	25	100	0	10
	12	90	19.5	10
ASTM soft	NA	100	23.0	10
Well control	NA	100	25.1	10

Table 10. Results of Cañon de Valle explosive analysis from the Phase III portion of this study. Detectable amounts of explosives were found only in samples from Cañon de Valle. In addition to the compounds shown below, the samples were analyzed for 1,3,5-trinitrobenzene, 1,3-dinitrobenzene, tetryl, nitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, 2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene, but these compounds were not detected. Water measurements are in $\mu\text{g/L}$ and sediment in $\mu\text{g/kg}$.

	date	HMX	RDX	2,4,6-TNT ¹	4-Am-2,6-DNT ²	2-Am-4,6-DNT ³
water	7/28/97	5.6	13.2	ND ⁴	0.5	1.1
	8/13/97	56.5	105	ND	19.6	15.7
sediment upper site	7/30/97	799	308	144	74.1	ND
	9/29/97	1130	1804	127	415	530
sediment lower site	7/30/97	366	ND	89.3	ND	ND
	9/29/97	91.2	104	26.2	352	345

¹2,4,6-trinitrotoluene

²4-amino-2,6-DNT

³2-amino-4,6-DNT

⁴below detection limit

Table 11. Reproduction and survival in *Ceriodaphnia dubia* exposed to pore waters collected in 1997 from Los Alamos National Lab streams. Asterisks indicate reproduction significantly different from the reference. N indicates the number of replicates; some treatments had less than ten replicates because of handling mortality during the test. Los Alamos 1b was tested on a separate test occasion, and compared to ASTM soft b, the control for that test.

Site	dilution (% original sample)	survival (%)	mean reproduction	N
Los Alamos Canyon 1 b	100	90	* 41.0	10
	50	80	33.4	10
	25	80	33.3	10
	12	80	23.8	10
Sandia Canyon	100	78	* 14.8	9
	50	90	30.2	10
	25	100	24.6	9
	12	80	* 13.0	10
Pajarito Canyon	100	100	31.5	10
	50	100	28.8	10
	25	100	28.6	10
	12	70	* 5.7	10
Cañon de Valle	100	100	31.3	10
	50	88.9	23.8	9
	25	90	28.6	10
	12	60	* 10.2	10
ASTM soft	NA	100	26.2	10
ASTM soft b	NA	90	26.1	10

Table 12. Reproduction and survival of *Ceriodaphnia dubia* in retest of 12.5% dilutions of porewater collected from Los Alamos National Lab streams. Two different dilutions waters are compared, ASTM Soft, and a reconstituted water of similar hardness made by diluting well water with reverse osmosis water (well soft recon).

Site	dilution water	survival (%)	mean reproduction	N
Los Alamos 1 b	ASTM Soft	80	23.8	10
	Well soft recon	80	32.1	10
Sandia Canyon	ASTM Soft	90	27.8	10
	Well soft recon	90	29.4	10
Pajarito Canyon	ASTM Soft	100	27.2	10
	Well soft recon	100	37.7	10
Cañon de Valle	ASTM Soft	100	25.1	10
	Well soft recon	100	43.7	9
ASTM Soft	NA	80	26.1	10
Well soft recon	NA	80	28.0	10

Table 13. Reproduction and survival in *Ceriodaphnia dubia* exposed to pore waters collected in 1996 from Los Alamos National Lab streams. This test was invalid due to the presence of males in the test, but replicates containing males were excluded from this analysis. Because of the problems with the test, no statistics were performed on these data. N indicates the number of replicates; most treatments had less than ten replicates because of handling mortality during the test and because of the exclusion of replicates containing male animals.

Site	dilution (% original sample)	survival (%)	mean reproduction	N
Los Alamos Canyon 1	100	100	32.8	6
	50	100	19.8	5
	25	71.4	10.7	7
	12	100	18.1	7
Sandia Canyon	100	88.9	16.7	9
	50	80	19.3	10
	25	100	21.9	7
	12	100	21.5	6
Pajarito Canyon	100	100	22.1	7
	50	100	20.7	9
	25	83.3	19.5	7
	12	100	15.3	7
Cañon de Valle	100	85.7	29.6	7
	50	100	16	2
	25	100	18.6	7
	12	80	13.2	5
ASTM soft	NA	100	29.6	5